A PHYLOGENETIC SURVEY OF MOLLUSCAN SHELL MATRIX PROTEINS

By

Michael T. Ghiselin

Systematics-Ecology Program, Marine Biological Laboratory
Woods Hole, Massachusetts

Egon T. Degens and Derek W. Spencer

The Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

and

Robert H. Parker

Systematics-Ecology Program, Marine Biological Laboratory
Woods Hole, Massachusetts

INTRODUCTION

Recent advances in biochemistry and cytology have vastly increased the availability of structures suited for phylogenetic research. Diverse and complicated chemical structures, such as cytochrome c (Margoliash and Smith, 1964) and chromosomes (Spencer, 1949), are amenable to a type of analysis based on formal properties analogous to that of traditional comparative anatomy. The growth of evolutionary biology has recently brought about a subtle, yet pervasive and fundamental, revolution in the aims and methods of systematics. The development of new techniques based on an understanding of the causes of evolution has so far been most pronounced in the study of species and speciation, but is being extended to higher levels (Cain, ed., 1959; Bock, 1959, 1960). The present work is an attempt to continue these trends, employing a new type of evidence made available by improved methods

of chemical analysis: the proteinaceous matrix which serves as a framework for the calcareous portion of the molluscan shell. The preliminary survey discussed here is a methodological experiment, evaluating the feasibility of new techniques (logical, chemical and statistical). It appears, from both the consistency of the results and reference to traditional comparative anatomy, that the approach used here is useful for classification. Until more data are collected, however, the detailed conclusions should be considered tentative.

The development of these techniques has depended upon an interdisciplinary approach which has allowed efficient use of the data that otherwise would have been impossible. Systematic methods and results are stressed in this account. A fuller discussion of the chemical aspects of the study is being published elsewhere (Degens, *et al.,* 1966). A publication (Degens and Spencer, 1966) giving raw data and details of analytical techniques and computer methods is available on request.

ACKNOWLEDGMENTS

ANATOMICAL AND CHEMICAL BACKGROUND

The molluscan shell (cf. Wilbur, 1964; Glimcher, 1960) is produced by secretion of precursors from the epithelial tissue in specialized areas of the mantle, and may consist of several layers. The outer layer, or periostracum, is not calcified; it is thought to consist of quinone-tanned protein (Brown, 1952). The inner

layers are calcareous, and include a proteinaceous matrix which serves as a nucleation site for specific cations and anions, and in which the mineral crystals are deposited. The crystalline structure (Bøggild, 1930) and protein configurations (Grégoire, 1961) are quite intricate, suggesting a highly organized system. In mollusks, the uncalcified matrix is laid down extracellularly; only after the matrix has been formed is inorganic material from the underlying tissue incorporated into the shell.

We have been guided by the hypothesis that the shell matrix is derived from at least one structural protein which originally had some function other than that which it now serves. It seems reasonable that the evolution of a new function has occurred gradually. With the change from a non-calcified to a calcified state, the protein should become increasingly reorganized, so as to facilitate the effective binding and interconnection of the parts. In general, this should involve a proportionate increase in the amount of those organic components which facilitate the binding of important ions and a decrease in the quantity of superfluous organic materials. There should also be changes in the type of cross-linkages that interconnect the individual parts of the protein. These hypotheses may readily be verified by their implications for systematics; they predict that certain types of correlations will occur between taxonomic groupings, environmental and physiological relationships, and chemical composition.

CHEMICAL METHODS

The shell material was separated mechanically from all extraneous proteins and after being ground to a powder, decalcified, in the presence of 10% trichloracetic acid solution, by hydrochloric acid, added in quantities stoichiometrically necessary to dissolve the calcium carbonate. The organic remains were centrifuged, washed, and hydrolyzed with 6N hydrochloric acid for 22 hours in vacuo. The amino acids were analyzed by automatic ion-exchange chromatography. The method gives reproducible results of better than 1% at the 10^{-8} molar level for identical runs. The overall variation of two identical samples from the same collection site is less than 3%; this figure includes the variation between specimens, and the errors introduced by decalcification, chromatography and data-handling. (For details, see Degens and Spencer, 1966.)

STATISTICAL METHODS

For the comparison of a few forms, visual inspection of the measurements for each amino acid is adequate to place the various

forms in sequences corresponding to hypothesized evolutionary lineages. With larger samples, however, the task of comparison is facilitated by statistical techniques using a computer. As certain amino acids tend to show a constant ratio relative to each other, they may be combined for the purpose of comparison. By means of a factor analysis, it was possible to reduce the data to a set of factor scores (8 in gastropods, 9 in bivalves), which explain 90% of the variance in the data. The factor scores may then be compared independently, or in an 8 or 9-dimensional hyperspace. We found that separate factor analyses for gastropods and bivalves were desirable, as with increasing phylogenetic diversity the various amino acids show slightly different patterns in their tendencies to vary in correlation with others. Our attempt to combine all the data for the phylum did little more than increase the number of extraneous relationships.

The statistical methods used here should not be confused with what has been called "numerical phenetics." Factor analysis, which has become the method of choice in psychology, but which has found little use in systematics, has several advantages over the clustering techniques employed, for example, by Sokal and Sneath (1963). A clustering technique tells nothing more than how "similar" things are, in terms of an arbitrary standard; what is called "overall similarity" is merely the summation of diverse properties which may be no more comparable than the shape and hardness of lightbulbs and pears. It is advantageous, when using any type of statistical technique, to work with a clearly formulated model. A factor analysis generates such a model, allowing the treatment of each individual factor as independent evidence, and its separate evaluation. It also enables one to formulate hypotheses and test them by reference to particular aspects of the data.

At first we tried to relate the species by considering each species as a point within the 8 or 9-dimensional factor space. The factor scores are the coordinates of the points. Species that have similar scores will be separated by only a short distance, while those with greatly different scores will be well separated. Table 1 presents the linear distances between each of the species in the factor space. This kind of comparison, although suggestive of relationships, was ambiguous and misleading. It only told how "similar" the material was, in terms of the new factors. On the basis of other evidence, it was obvious that certain clearly unrelated forms were grouped close together. This is readily understood, for the different amino acid variations are not strictly commeasurable, and

the values give only sequences. That is, the ordering is an intensive, not an extensive one (Ghiselin, 1966a). It is not surprising, therefore, that the summation of similarities grouped together: 1) unrelated forms which had changed little from the ancestral state; 2) unrelated forms which had undergone the same change to an extreme degree; and 3) "advanced" forms which had attained the same grade through parallelism.

Yet by taking the series of factor scores as independent evidence, and arranging the species in series which show changes for each factor in a particular *direction,* it was possible to arrange the various forms in series corresponding to divergent genealogical lineages. The forms previously misplaced then fell into more reasonable positions, and it could be demonstrated that the earlier misplacement had been due, say, to great alteration, in diverse lineages, of one particular factor. It was possible to verify these inferred relationships by means of comparative anatomical evidence. Our data are here presented (Tables 3 and 4) in the form of series showing progressive changes in factor scores. The series correspond to a system of inferred phylogenetic relationships, diagrammed as a phylogenetic tree shown on the left, with the direction of trends shown by arrows. The phylogenetic trees are strictly genealogical; distances mean nothing. The diagrammed arrangements are those which are most strongly supported by our amino acid evidence, and reasonably consistent with comparative anatomy. As a number of alternative interpretations are possible, the reader may wish to evaluate them. This can easily be done by writing out the factor scores on paper, and cutting out strips giving the scores; these may then be placed in any desired sequence.

SYSTEMATIC METHODS

Although we have stressed supra-generic relationships, future work will probably depend on precise species identifications. Therefore we have taken the precaution of figuring (Plates 1, 2) representatives of most of our samples, and have drawn upon materials deposited in the Museum of Comparative Zoology.

Future work may have to incorporate a larger and more rigorously selected sample than does this preliminary study, in order to account for variation. We have selected several specimens from widely separated localities and different ecological conditions, and find that there is considerable temperature-dependent variation (Degens *et al.,* 1966). The particular type of variation that occurs, however, appears to be different for each species. Some

evidence that convergences occur when there is a change from marine to freshwater or terrestrial habitats will be discussed below. The environmental effects can be considered "noise" in evaluating genealogical relationships. If we could remove some of the environmental effects, perhaps some of the anomalous relationships could be resolved. The removal of these effects would require accurate environmental data for each species, selected for a wide range of conditions, and, preferably, experimental studies.

To some extent, this study is based upon traditional comparative anatomical methods. The material studied consists of highly-structured proteins, the parts of which have relational properties such as allow one to abstract a common type from which all may be derived. To be sure, we do not know the details of structure, but our techniques do allow us to infer that different changes have taken place, and to correlate these with each other. However, our argument is not limited to such formal comparison, as our hypothetico-deductive system includes premises about chemistry and evolution which affect the results.

The hypothetical biochemical explanations outlined above imply that there will be a gradual diminution in the quantity of carbohydrate and protein in correlation with evolutionary development. At the same time, there should be a general trend toward increase in chemical groupings useful for nucleation (e.g., aspartic acid) and for cross-linkages (e.g., phenolic compounds). Further, forms which retain a high amount of protein should be those with a structure which may readily serve as a precursor for more modified forms. The primitive condition may therefore be inferred on the basis of physiological criteria, providing independent evidence for inferences based on divergent specialized types. We do not, however, presuppose an overall "primitiveness" for any particular organism. Different structures may evolve at different rates, and the ancestral form of a group may be reconstructed with rigor only when the hypothesized properties of each system are supported by empirical evidence. This point is crucial in the present study, which shows that there is no strict correspondence between the degree of evolutionary advance in the shell and in other structures.

Perhaps the major difficulty for any phylogenetic study is parallelism, and the present study is no exception. Evolutionary changes may tend in the same direction, and occur repeatedly. When this happens, a grouping together of similar forms on the basis of such changes leads to a series of grades rather than clades. It is

abundantly clear in bivalves, for example, that some of the structural changes (in hinges, gills, stomachs, etc.) used as phylogenetic evidence have evolved a number of times. Classifications erected on only one such line of evidence take it as *a priori* that some character can only have evolved once (e.g., Purchon, 1959). Such premises are not only without support, but are flatly contradicted by analogy with such polyphyletic structures as the mammalian ear-bones. If a structure evolves once, it can evolve repeatedly. The problem of parallelism has been de-emphasized by Remane (1956), and Sokal and Sneath (1963), on the grounds that sometimes there is divergence rather than parallelism. This misses the point; there is no way to estimate the probability for a structure to evolve in more than one direction.

A solution to the problem of parallelism has been proposed by Bock (1959, 1960) and has found some application by one of us (Ghiselin, 1966b) in gastropod phylogeny. To understand the technique, one must realize that it entails a shift in emphasis from observed characters to a model of divergent genetic and anatomical potentialities. It is assumed that however many times a character develops, closely related lineages *may* evolve in different directions; this follows from similarities and differences inherent in the genetic potentialities within each lineage. If the forms can be placed in at least two sequences corresponding to divergent tendencies (rather than parallel stages), then the arrangement supports the view that each member of one divergent group is not related to any member of any other such group. From placing them in sequences which do not diverge, but proceed to only one derived state, no systematic inferences can be drawn, as any number of changes would explain the facts; founding groups on lack of divergence in a derived trait is therefore the logical fallacy of denying the antecedent in a conditional statement. In other words, the development of a character is not evidence as such for relationship to a form also having that character, but only that both of the two forms having it are not related, for the same reason, to a form having some different alternative. For example: Purchon (1958, 1959) has distinguished five types of stomachs in bivalves. Three of these occur in the "higher" bivalves studied here: types III, IV and V. Both III and V may be derived from IV, and the advanced types overcome certain functional inefficiencies in the ancestral form. If the bivalves are divided into groups, including one for forms with type V stomachs, and one for those with type III, reference to other kinds of evidence demonstrates that forms within each grouping are most closely related to forms with type

IV. Therefore, the changes from IV to V, and from IV to III have occurred more than once. Nonetheless, a system of relationships which abstracts one group with type IV and only type V, and another with type IV and only type III, fits in quite well with other evidence (cf. Newell, 1965). The biochemical evidence given here likewise supports this division as indicative of relationships. A valid phylogenetic method, then, admits any number of uni-directional changes, as IV to III or IV to V; a contradiction arises only when the divergent, derived forms (III and V) both occur within more than one lineage. Such a shift in emphasis may seem minor, especially when the logic of conditional statements is over-looked, but it seems to resolve much of the confusion that has resulted through efforts to erect systems of classes differentiated by visible, intrinsic properties, rather than corresponding to the more fundamental genetic and evolutionary order. Classification, in other words, involves, not simply grouping organisms on the basis of resemblances, but sorting them out into groups which differ in their programmed genetic information, and therefore have divergent potentialities and tendencies, irrespective of whether these at any moment are realized or not.

PHYLOGENETIC SURVEY

Ancestral models, Amphineura, Cephalopoda, Monoplacophora. By analogy with other studies on protein evolution, one might assume that differences in the matrix protein have resulted from replacement of particular amino acids owing to corresponding chromosome mutations affecting the template. Such modifications may underlie some of the diversity, but we feel that the major reason is to be sought elsewhere. Chemical and electron microscope studies (for references cf. Degens *et al.,* 1966) indicate that the protein is heterogeneous. Solubility tests (Degens and Spencer, 1966) suggest that the protein of *Mercenaria* resembles collagen in solubility and molecular weight, although our quantitative data show differences in amino acid ratios; for example, the molluscan shell matrix differs from its analogue in vertebrate bone, in lacking the hydroxyproline characteristic of collagen.

Some amino sugar is present in both shell and mantle of mollusks. The amount of carbohydrate in the shell varies considerably throughout the phylum. The relative proportion of carbohydrate to protein seems to have decreased progressively with functional improvement in shell structure; simultaneously, the percentage of protein has decreased, but at a different rate. These

trends may be explained if one hypothesizes that the shell was produced from a protein-carbohydrate complex which has been modified so as to improve nucleation. Such a change has occurred in calcified arthropod integuments (Rudall, 1963; Carey *et al.*, MS), which show a decrease in the proportion of protein and an increase in the amount of hexosamine with calcification. In gastropods and bivalves, on the other hand, amino sugars decrease greatly. The integument of arthropods and mollusks may be derived from a common ancestral precursor (presumably originating at an annelidan stage of evolution) in which a non-calcified protein was linked to carbohydrate. In arthropods, the amino sugars have evidently been retained, while protein has been so altered as to expose the acidic and basic side-chains responsible, respectively, for concentrating calcium and carbonate ions. Mollusks differ in having lost much of the amino sugar, while elaborating the protein.

Our data suggest that the molluscan shell has been evolved by modification of proteins which occurred in the mantle. Reference to Table 2 shows the type of relationships and evolutionary changes that seem to characterize the phylum. Here comparisons are given of the amino acid ratios in both mantle and shell of a chiton (*Chaetopleura*), the shells of several cephalopods, representative "primitive" gastropods and pelecypods, a monoplacophoran (*Neopilina*), and the calcified and non-calcified integuments of portunid crabs. The relationships shown by this table are, at first glance, ambiguous. It may be seen that certain amino acids are more (or less) abundant in the shell than in the mantle of *Chaetopleura*. Often, these particular amino acids occur in about the same concentration, as in *Chaetopleura, Spirula* and *Sepia,* whereas in *Loligo, Nautilus* and other mollusks, the range is distinctly different (e.g., aspartic and glutamic acids, perhaps glycine and alanine, and cystine). This relationship is explicable in terms of a high degree of calcification, without loss of carbohydrate, in *Chaetopleura, Spirula* and *Sepia.* Perhaps *Loligo* somewhat resembles *Nautilus* because although it is rich in carbohydrate, it is not highly calcified. In a number of other amino acids, however, *Loligo* is (in correlation with the high ratio of carbohydrate to protein, but independent of calcification) close to *Chaetopleura, Spirula* and *Sepia,* and most distinct from *Nautilus, Haliotis, Nucula* and *Mytilus* (threonine, proline, perhaps glycine and alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and perhaps hydroxylysine). Of these, only four (threonine, methionine, tyrosine and phenylalanine) would seem to correlate with

the high level of carbohydrate in *Chaetopleura, Spirula, Sepia* and *Loligo.* Any attempt to give phylogenetic interpretations to these relationships without weighting would be foredoomed to failure. *Nautilus,* which retains many primitive traits, more closely resembles members of other classes than its closest relatives. The only cephalopod much like it is *Loligo.* But *Loligo* is, in a way, intermediate between *Nautilus* and the other cephalopods in just those characters in which the latter resemble the Amphineura. There must have been convergences.

The ambiguity may be resolved if one supposes that there have been three, largely parallel, stages in the evolution of the shell. *First,* there would be the development of a calcified, but carbohydrate-rich shell. *Chaetopleura, Spirula* and *Sepia* would represent this stage. *Loligo* would retain much of the character of the first stage, but have lost (or conceivably never have developed) some of the functional units related to calcification, while retaining others significant in carbohydrate physiology. *Second,* there would be a loss of carbohydrate. This would explain the difference between *Nautilus* and other cephalopods, and, as a convergence, the resemblances between *Nautilus* and *Haliotis,* etc. *Third,* there would be an elimination of that part of the protein which is superfluous as a structural element or in calcification, and the development of more efficient means of cross-linkage. These three stages would occur more or less in parallel fashion, perhaps simultaneously, but a stepwise sequence would explain such evident gaps as that between *Nautilus* and other cephalopods.

Within the Cephalopoda, the shell of *Nautilus* is the most advanced in its chemical evolution, in sharp contrast to its many primitive anatomical features. The other cephalopods would appear to have survived in spite of a poorly-developed shell, largely by converting it to an endoskeleton. The *Chaetopleura* shell is of equally low chemical grade; there is no reason to think that this has occurred by degeneration. *Neopilina* belongs to the class Monoplacophora, thought to be ancestral to many other classes of mollusks, but it does not show any close agreement in amino acid ratios to any other mollusk. However, as we were unable to separate all the periostracum from our sample, the data are hard to interpret. On the other hand, the proportions of carbohydrate, protein and mineral show that the shell of *Neopilina* has undergone considerable modification.

It appears that the major, early steps in shell evolution (calcification, loss of carbohydrate) are parallel and unidirectional. Therefore, while of physiological interest, they are uninformative for

cladal phylogeny. In later stages, however, many divergences oc-
cur, and these are most useful in establishing genealogical rela-
tionships. Such changes may be largely due to different kinds of
cross-linkages. These are primarily covalent linkages, such as:
1) various linkages to carbohydrate moieties; 2) disulfide connec-
tions; 3) unusual amino acids (e.g., desmosine and isodesmosine);
4) phenolic compounds and quinones (for details, see Degens
et al., 1966). The matrix seems to be secreted as soluble pre-
cursors which, at about the time of secretion, are acted upon by
enzymes and other substances which produce the cross-linkages.
Differences in composition may result from variations in the rates
and kinds of such processes of secretion and modification, as well
as from differences in the raw materials. The subsequent discus-
sions of gastropod and bivalve shell evolution trace out progressive
changes in various groups; these changes evidently are related to
different kinds of secretory processes and materials.

Gastropoda. Table 2 shows the raw data for *Haliotis,* a form
which approximates a hypothetical ancestral gastropod. Changes
within the class are shown in Table 3. As would be expected
from the usual classification of the order Archaeogastropoda, *Hali-
otis* (superfamily Zeugobranchia) and *Astraea* (superfamily Tro-
chacea) are quite similar. But *Fissurella,* another zeugobranch,
is quite distinct from *Haliotis;* as it shows no clear relationship to
any other group, the difference is probably due to divergence. The
superfamily Patellacea, as shown by *Acmaea,* is likewise distinct
and isolated. The representative of the other archaeogastropod
superfamily, Neritacea (*Nerita*), resembles *Viviparus,* a form usu-
ally placed in the order Mesogastropoda. Both *Nerita* and *Vivi-
parus* are in some ways transitional between the Archaeogastro-
poda and Mesogastropoda; the resemblance may be convergent,
but there is no compelling reason to think that it is.

The rest of the gastropods studied break down into three major
groupings which agree fairly well with generally recognized natu-
ral groups. The less-modified members of each group are quite
similar, indicating a close relationship and common origin.

The first of these groups (Table 3, *Crepidula* to *Murex*) in-
cludes the order Neogastropoda as a distinct sub-group and most
of the Mesogastropoda (excepting only some forms known to
have opisthobranch affinities). The Neogastropoda (*Colus, Nas-
sarius, Urosalpinx* and *Murex*) show distinctive tendencies in the
modification of several factors, although the original pattern is not
far removed from that of mesogastropods. This fits in with other
biological data; the neogastropods are distinct but not greatly

modified in terms of morphology, except for the more specialized members of the group. But neogastropods are distinct in chromosome number (Nishikawa, 1962) and evidently in many physiological characters also. The superfamily Naticacea (here, *Lunatia* and *Polinices*) has a pattern of change clearly distinct from other mesogastropods, and proceeding in a direction opposite to that of, say, *Littorina* and *Cypraea*. One of the unexpected results of this study was that *Melanella* has the same tendencies as occur in the Naticacea. *Melanella* has been placed in the superfamily Aglossa, which may be an artificial assemblage of parasitic forms with tall shells. An affinity of *Melanella* to the Pyramidellidae (now grouped as opisthobranchs), as suggested by Thiele (1935), is not consistent with our findings; similarly, *Melanella* lacks the characteristic reproductive system and larval shell of opisthobranchs.

Littorina, which, along with other members of the superfamily Littorinacea, is often thought to stand near the base of the mesogastropod series, has a pattern of change which tends in the direction characteristic of *Cypraea* and *Crepidula*, fitting in well with the concepts of Thiele (1935). An affinity between the Cypraeacea (*Cypraea*) and Calyptraeacea (*Crepidula*) follows from intermediate forms (Lamellariacea) (cf. Fretter and Graham, 1962). The type of variation that occurs within a genus may be seen in *Crepidula*. The range is fairly narrow and in conformity with overall trends, but some of the factors appear to have changed more than others. Such variation clearly indicates that a larger sample will be necessary in future work, but the utility of the method stands.

Another distinct grouping of mesogastropods consists of *Turritella* (Cerithiacea) and *Epitonium* and *Janthina* (Ptenoglossa). Both superfamilies differ from all other gastropods in possessing a curious, spermatophore-like structure, the spermatozeugma. The Cerithiacea are of disputed taxonomic position, often looked upon as transitional between various major lineages of mesogastropods and opisthobranchs; our data suggest a closer affinity to opisthobranchs. However, Cerithiacea and Ptenoglossa also have much in common with Neogastropoda, and the basal members of each of our three main groupings (*Architectonica, Turritella, Littorina*, etc.) are quite similar. Therefore, the precise cladistic relationships of each group remain uncertain.

The third group corresponds to the Euthyneura, or the subclasses Pulmonata and Opisthobranchia. The Euthyneura are characterized by hermaphroditism, a tendency to loss of the effects of

torsion, a distinctive type of spermatozoon, a peculiar ctenidium, and a heterostrophic larval shell (except where secondarily modified). Fretter and Graham (1949) have placed the family Pyramidellidae in this group. As *Architectonica* has a heterostrophic larval shell, Robertson (1963; also, Robertson and Merrill, 1963) and others have argued for its close affinity to the Euthyneura, and this placement is adopted here.

Some workers have advocated abandonment of the subclasses Pulmonata and Opisthobranchia (cf. Taylor and Sohl, 1962). However, this has been done solely on the basis of highly speculative assertions that Pulmonata is a polyphyletic assemblage; there is no real evidence for this view (cf. Ghiselin, 1965, 1965b).

In its low protein content, and in the amino-acid ratios, *Architectonica* is an excellent precursor for the opisthobranchs and pulmonates, the members of each of which can readily be derived by modification of different factors. This fits in with the larval shell types. Chemically, *Architectonica* differs but slightly from such Cerithiacea as *Turritella*. Indeed, Thiele (1929) put *Architectonica* in the Cerithiacea. On the basis of fossil evidence, Knight, Batten and Yochelson (1954) relate the superfamily Ptenoglossa to the Pyramidellidae and group both with the opisthobranchs. Clench and Turner (1951) likewise stress the opisthobranch affinities of Ptenoglossa. Thus the close relationships between these groups, on the basis of other evidence, supports the inference from shell biochemistry.

The general conclusions reached by one of us on the basis of various lines of evidence on the relationships of opisthobranchs (Ghiselin, 1966b) are fairly well borne out by the present study. *Bulla* shows a beginning in the development of trends characteristic of the Anaspidea (*Akera, Aplysia*); gizzard morphology and anatomy generally support this relationship. Both *Akera* and *Oxynoe* are distinguished from all other mollusks by the presence of hydroxyproline in the shell. This amino acid is distinctive of collagen; its adaptive advantage is probably one of making the shell flexible, and the same explanation suffices for the rather high protein content. Perrier and Fischer (1911) describe a muscle inserted near the rim of the shell in *Akera* which bends the shell and closes the mantle cavity. An evidently homologous muscle provides a respiratory current in *Cylindrobulla,* a form transitional between Anaspidea and Sacoglossa (including *Oxynoe*) (cf. Marcus and Marcus, 1956); from it may be derived the shell adductor muscle in the bivalved gastropod (Baba, 1961). These homologies,

both morphological and chemical, strongly support the relation-
ship previously inferred (Ghiselin, 1966b) on the basis of repro-
ductive anatomy and spermatozoon morphology. Although both
Aplysia and *Dolabella* are universally looked upon as closely
related to *Akera,* neither contains hydroxyproline in its shell. *Aply-
sia* is closest to *Akera,* but deviant, and *Dolabella* is so distinct
that no particular relationship for it follows from our data. Al-
though Table 3 shows *Akera* and *Oxynoe* more closely related to
each other than to *Aplysia,* which is a possibility, it seems more
reasonable to infer that *Aplysia* and *Dolabella* have lost their
former resemblances to *Akera;* their shells are vestigial and no
longer function in the adults. *Cavolina* (order Thecosomata) and
Umbraculum (order Notaspidea) are here grouped together on
the basis of a few similarities, especially factor 4; however, to
place *Cavolina* with the *Akera-Bulla* group, as previously sug-
gested on the basis of admittedly weak anatomical evidence (Ghi-
selin, 1966b), would be fairly consistent with our data.

Insufficient information was available to establish relationships
for the basommatophorous Pulmonata (*Siphonaria, Helisoma* and
Planorbis), beyond relating them to other Euthyneura. In the Sty-
lommatophora, on the other hand, the relationships seem clear:
Achatinella (suborder Orthurethra) and *Succinea* (suborder Hete-
rurethra) are very close. Admitting the possibility of convergence
due to a terrestrial habitat, our data contradict the view of Rigby
(1965) that *Succinea* is an opisthobranch with affinities to the
Anaspidea (*Akera, Aplysia,* and *Dolabella*). The pulmonate na-
ture of *Succinea* is further supported by its stylommatophoran
chromosome morphology (Inaba, 1959), gizzard type, nervous
system, and reproductive morphology and cytology.

Bivalvia. The relationships of bivalves have long been contro-
versial, owing to parallelism, convergence, and an insufficient num-
ber and variety of characters. (For a useful summary of the data
which have been used, see Newell, 1965.) Hinge-teeth (Dall, 1894,
and others) have been very useful, especially with fossils, and are
particularly useful in that some types are divergent, but some
convergences have taken place. The attempts of Pelseneer (e.g.,
1911) to divide the bivalves on the basis of gill elaboration result
in partially artificial groupings, because of unidirectional, parallel
evolution. There are analogous difficulties with palp types (Stasek,
1963), kidneys (Odhner, 1912) and the pallial sinus. Douvillé's
(1912) division into normal, sedentary and burrowing branches
does show fair correspondence to other groupings, and this is to

be expected, as the branches are divergent. Again, certain spe-
cializations for which there seems to be no unidirectional trend
give groupings which correlate with other types of evidence: cer-
tain types of cilia on the gill (Atkins, 1938), and the anisomyarian
state. We have already alluded to the value of some modified and
divergent stomach types (Purchon, 1958, 1959). When the
available evidence is properly weighted — emphasizing divergences
and recognizing possible effects of parallelism and convergence —
the bivalves fall quite readily into natural groupings which may be
supported on the basis of various kinds of evidence. The same
is true of the new information summarized here. Although the pre-
cise sequence of genealogical relationships remains uncertain, the
general pattern obtained correlates quite rigorously with other
types of evidence. Indeed, our groupings correspond almost per-
fectly with the classification proposed by Newell (1965), except
that our data (and some other evidence) suggest removing one
group to another, closely related one.

Our data are presented as factor scores in Table 4. *Nucula* may
be considered as having properties closest to the common ancestor
of the class. We give no starting point for the tree because the
data are consistent with a variety of interpretations, although the
common stem presumably is near to *Nucula*.

Nucula, Solemya, Malletia and *Yoldia* are members of the
order Protobranchia, a group generally held to be representative
of the ancestral bivalve stock. The placement of *Malletia* and *Yol-
dia* close together, distinct from *Nucula* is in agreement with the
usual classifications (McAlester, 1964). *Solemya* is similar to
Nucula but somewhat modified, and its relationship to the Nucul-
anidae (*Malletia, Yoldia*) is only weakly supported by our data.
Newell's (1965) removal of *Solemya* to a taxon of rank equal to
that of other protobranchs is perhaps based on an overemphasis of
shell morphology.

Periploma and *Lyonsia* are not greatly altered from the *Nucula*
stage. The pattern of modification agrees well with their usual
classification as Pandoracea, and with the view that they are but
distantly related to the other major groups of higher bivalves in-
cluded in this study. *Neotrigonia* is of uncertain relationship. The
position shown is only weakly supported by our evidence. Indeed,
it would in many ways be more satisfactory to relate it to the
Arcidae (*Anadara* and *Limopsis*) as suggested by Odhner (1912)
and others. It shows a pattern of change which is intermediate be-
tween *Mytilus* and *Anadara* in factors 3, 4, 5, and 9; only factor 1
is out of place, and this could be a divergent or ancestral state.

The connections shown on the diagram for *Anadara* and *Limopsis* serve only to suggest a possible relationship of other bivalves to one lineage: the two genera are closely related. A close relationship of the Arcidae to the Anisomyaria (*Mytilus, Crassostrea* and *Aequipecten*) is supported by the presence, in the Arcidae, of micro-laterofrontal cilia, which are common in Anisomyaria (Atkins, 1938), and also by the type III stomach (Purchon, 1957) which occurs only in Pteriomorpha (*Mytilus* and *Crassostrea,* but not *Aequipecten*).

The remaining bivalves are Heterodonta (*sensu* Newell, 1965). *Pitar, Mercenaria, Saxidomus* and *Petricola* are all members of the Veneracea, and clearly form a natural group, as do *Corbicula* and *Arctica* (Corbiculacea). The placement of *Macoma* and *Tagelus* (Tellinacea) with *Mulinia* and *Laevicardium* is in agreement with Newell's (1965) classification, but inclusion of the Corbiculacea in this grouping is not. However, *Mulinia* and some of its allies share with the Corbiculacea a desmodont hinge, unlike the Veneracea; the hinge of the Tellinacea is likewise aberrant, although *Laevicardium* (here placed as an early offshoot of the Tellinacea-Corbiculacea line) is heterodont.

NOTES ON ECOLOGY

Invasion of land and fresh water. In the present study we have, on the whole, attempted to hold the environment constant by selecting our material from similar habitats. An exception is a few freshwater and terrestrial forms. The effects of such a drastic change in habitat are striking. In Table 5 are compared two fairly closely related prosobranchs, the marine *Nerita* and freshwater *Viviparus,* and a similar series of two marine Euthyneura (*Architectonica* and *Siphonaria*), two freshwater pulmonates (*Helisoma* and *Planorbis*) and two land pulmonates (*Succinea* and *Achatinella*). In general, the same amino acids change in the same direction with shifts to both land and fresh water: aspartic acid (factor 8); threonine, glutamic acid, glycine, methionine (factor 1); perhaps histidine (factor 3); proline, arginine (factor 4); serine, alanine, phenylalanine (factor 6). It holds true as a general rule, that the change in concentration for these amino acids is in the direction of the concentrations that prevail in more primitive mollusks such as *Haliotis* and *Nucula* (Table 2). Other amino acids either remain constant or change in the opposite direction in terrestrial forms: valine, isoleucine and leucine, i.e., factor 2. Tyrosine (factor 3) seems to change in the direction away from

the primitive state; the freshwater forms change to a greater degree than the terrestrial ones. The other members of factor 3 (hydroxylysine, lysine and histidine) show no clear-cut pattern, but tend to approach the primitive level. Tyrosine and phenylalanine seem anomalous, but this may be due to their both being phenolics. Although we do not have enough data to obtain conclusive results (especially since we do not have several lineages of terrestrial forms), the evidence suggests that convergent changes occur in freshwater and terrestrial environments. It is probable that the shift to a new habitat makes it advantageous to concentrate the same functional units which had been decreased, in proportion, among advanced marine forms. Such a pattern of change is consistent with the presumed heterogeneity of the protein. When the structure and function of the substances underlying the variation are known, it should be possible to explain just why particular changes take place.

Effects of salinity and temperature. The sample given here is large enough only to be suggestive, but there is reason to think that within a species, salinity and temperature may have some effect on amino acid ratios. However, the effect seems to be characteristic for each species, and there is little evidence that particular amino acids vary consistently in correlation with salinity or temperature for larger taxa as a whole. Hare (1962) measured the amino acid content for *Mytilus californianus* over a wide range of temperatures, and found no correlated change in protein composition. Comparison of other species (*M. edulis* and *M. viridis*) from widely different habitats supports the same view (Degens and Spencer, 1966). However, the periostracum of *Tagelus divisus* is distinctly different in forms from Bermuda, on the one hand, and Nantucket and Long Island, on the other (Degens *et al.*, 1966). A factor analysis of the shell matrix proteins in *Polinices duplicatus, Mulinia lateralis* and *Anadara transversa* (Degens *et al.*, 1966) shows that there is a distinct correlation between mean temperature and salinity and certain of the factors used in establishing genealogical relationships. *Mulinia* and *Anadara* both showed the same direction of change in factors 1 and 5, but only *Mulinia* changed progressively in factor 8, while *Anadara* changed in factors 2, 6 and 9. *Polinices* changed in the opposite direction in the threonine-glutamic acid-glycine factor. For *Mulinia,* a multiple regression analysis was run to evaluate the degree of predictibility of each amino acid from the following environmental parameters: median temperature, range of temperature, median salinity, range of salinity, and depth. These analyses showed that only in the case of isoleucine, leucine, valine and cystine, was a statistically significant

linear regression obtained. For example, 82% of the variance of isoleucine is explained in terms of the environmental parameters. Of these, the median temperature and range of salinity were by far the most important, accounting for *ca.* 45% and *ca.* 30% of the variance, respectively. Such temperature dependent variation as occurs may reasonably be attributed to differential effects of temperature on the various chemical processes involved in shell deposition. Some of the patterns of variation may ultimately be related to genetic differences and therefore cast light on the evolution of the underlying physiological mechanisms. The precise significance of such variation must await the accumulation of a sufficient body of relevant data, but the problem is under investigation.

CONCLUSIONS

Success in grouping mollusks on the basis of shell matrix proteins suggests that the method may prove useful in the future. However, the precise placements given by this study must remain speculative until more adequate samples and better techniques are available for dealing with ecological variation and other problems. Preliminary results (Degens *et al.*, 1966) indicate that periostracum also is useful for phylogenetic inference, and other tissues show promise.

In any such study as this, it is essential that rigorous methodology be employed. This is clear-cut evidence that various lineages have undergone the same changes repeatedly and independently. Because of mosaic evolution, the rates at which changes occur in a single lineage are not necessarily the same for different structures. It is both bad biology and fallacious logic to place whole organisms in a series from "primitive" to "advanced" forms and argue that one has a real series of genealogical relationships. The term "primitive" is correctly used in a descriptive sense, in referring to the earlier stages of an evolutionary sequence. In such comparisons it is perfectly valid to assert, for example, that the noncalcified molluscan shell is more primitive than the calcified. But such comparisons give only intensive sequences: it is nonsense to measure "primitiveness" by summing up the degree of advancement in several different systems, as the various comparisons are not in commeasurable units. The present study illustrates this point very well. In terms of the degree to which metamerism has been lost, one could rank some mollusks thus: *Neopilina; Nautilus; Chaetopleura; Sepia; Haliotis* and *Nucula.* In terms of eye anagenesis, the

order would be: *Neopilina* (?); *Chaetopleura* and *Nucula; Haliotis; Nautilus; Sepia*. For shells it would be: *Chaetopleura* and *Sepia; Nautilus, Haliotis* and *Nucula; Neopilina*. The absurdity of trying to elaborate a calculus of evolutionary advance should be obvious. Nonetheless, one often reads assertions that a particular relationship should be rejected because modern members of some group are not "primitive" enough for some of their characters to represent the ancestral state. The present confusion in molluscan taxonomy may be expected to continue as long as such fallacy is perpetuated.

The approach has obvious use in developing a comparative biochemistry and physiology of calcification. The matrix is sometimes preserved in fossils, and study of its paleontology has already begun (Degens and Love, 1965; Degens and Schmidt, 1966). The effects of environmental factors should be particularly interesting. A change to fresh water from the sea would affect the pH and various ion concentrations, as is suggested by the rather high protein levels in freshwater forms. Where the calcification mechanism is inefficient, and where the medium in which calcification occurs is not regulated, slight changes in the environment — ionic ratios, pH, temperature, etc. — might have great effect on biota. Conceivably, physical and chemical changes in the environment might help to explain the greater abundance of fossils in Cambrian and younger rocks than in older ones, and mass extinction of ammonites and nautiloids. It is well known that the formation of coral reefs is dependent on temperature, and it seems a reasonable analogy that the success or failure of many organisms may be intimately connected with the process of calcification.

SUMMARY

1. Measurements of the amino acids in the shells of selected mollusks show a wide diversity. Some differences may be related to progressive evolutionary development of the shell.

2. Factor analysis shows that 90% of the variance may be explained in terms of a few factors. A technique which overcomes problems of parallelism and convergence has been developed for inferring phylogenies on the basis of differences in the factor structure.

3. Various phylogenetic hypotheses and classification schemes are evaluated on the basis of the evidence obtained in the study. The results are in close agreement with conventional classification systems, and cast some light on the positions of forms of disputed relationship.

4. Preliminary results show that there are convergences with shifts from marine to freshwater habitats, and that temperature and salinity dependent variation poses a considerable, but not insoluble, problem of interpretation.

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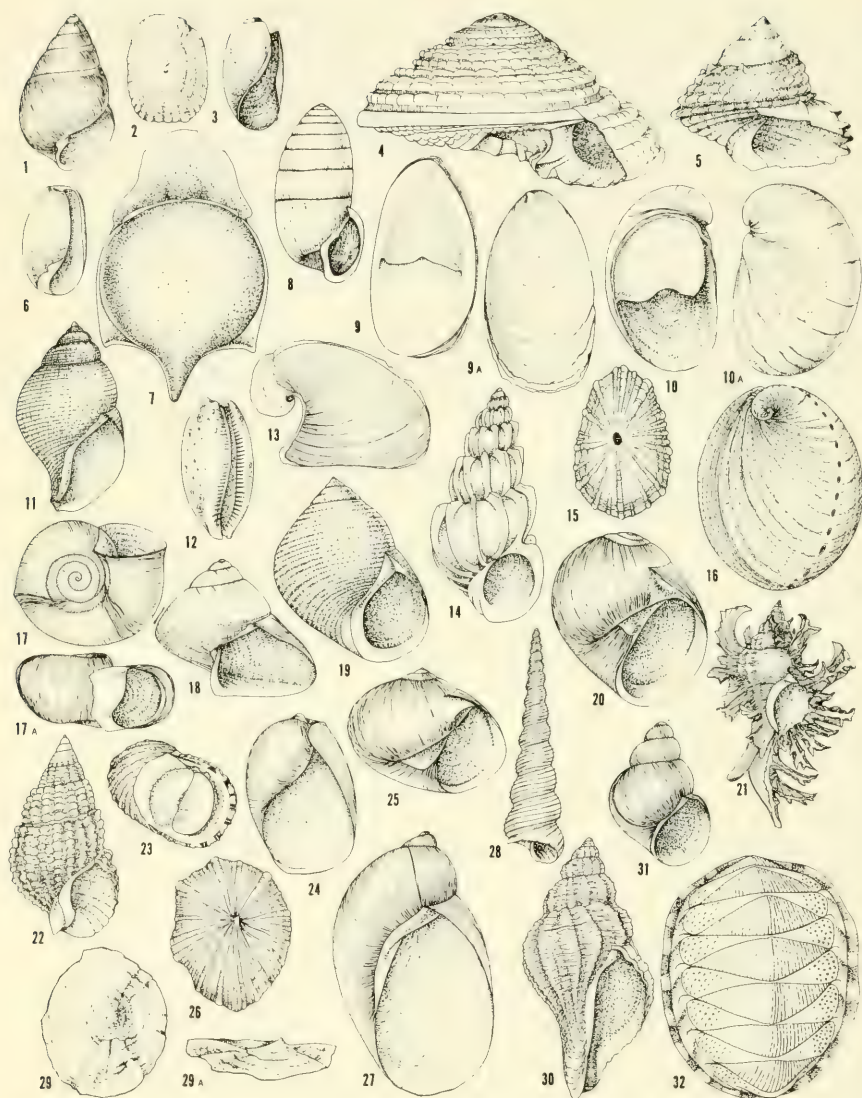
PLATE I

GASTROPODA

Figure 1. *Achatinella lorata* Pfeiffer, 1848, Oahu, Hawaiian Islands, Pease collection, MCZ (Mollusk Department) — aperture view. X 1.

Figure 2. *Acmaea pustulata* (Helbling, 1799), Puerto Sosya, Dominican Republic, MCZ, coll. 1937 — top view. X 2/3.

Figure 3. *Akera soluta* (Gmelin, 1791), Zanzibar, MCZ No. 4444 — aperture view. X 1/3.

Figure 4. *Architectonica nobilis* (Roding, 1798), Middle Atlantic Coast, 2-10 m, MCZ No. 90867 — aperture view. X 2.

Figure 5. *Astraea caelata* (Gmelin, 1798), Pelican Shoals, Florida, MCZ, coll. 1939 — aperture view. X 2/3.

Figure 6. *Bulla striata* (Bruguiere, 1792), Puerto Vieja, Dominican Republic, MCZ, coll. R. H. Parker, April 1965 — aperture view. X 2/3.

Figure 7. *Cavolina tridentata* (Forskal, 1776), pelagic, West Indies, Alexander Agassiz coll. 1879, MCZ (uncat.) — aperture view. X 2.

Figure 8. *Cerion regium* (Benson, 1849), northeast end of Castle Island, Crooked Island Group, Bahamas, B.W.I. — coll. Robertson and Scott, 1958, MCZ (uncat.) — aperture view. X 2/3.

Figure 9. *Crepidula fornicata* (Linné, 1767), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-2211-65, Systematics-Ecology Program, Marine Biological Laboratory — interior view; 9A, exterior view, X 1 1/3.

Figure 10. *Crepidula plana* (Say, 1822), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-275-65, Systematics-Ecology Program, Marine Biological Laboratory — interior view; 10B, exterior view. X 2/3.

Figure 11. *Colus trophius* (Dall, 1919), off San Francisco, California, 1874-1929 m, coll. R. H. Parker, MCZ (uncat.) — aperture view. X 1 1/3.

Figure 12. *Cypraea zebra* (Linné, 1758), Miami, Florida, MCZ (uncat.) — aperture view. X 1/3.

Figure 13. *Dolabella callosa* (Lamarck, 1801) = *scapula* (Martyn), Calapan, Mindoro, Philippine Islands, MCZ No. 96107 — side view. X 2/3.

Figure 14. *Epitonium angulatum* (Say, 1831), near mouth of New Brazos River, Freeport, Texas, MCZ No. 230893 — aperture view. X 2.

Figure 15. *Fissurella barbadensis* (Gmelin, 1791), Romey Point, Bonnguen Air Force Base, Puerto Rico, MCZ No. 1956 — top view (exterior). X 2/3.

PLATE I (Continued)

Figure 16. *Haliotis cracherodi* (Leach, 1814), San Diego, California, MCZ No. 58659 — exterior view. X 1/3.

Figure 17. *Helisoma trivalvis* (Say, 1817), La Porte, Indiana, MCZ No. 63234 — side view; 17B, aperture view. X 1 1/3.

Figure 18. *Janthina janthina* (Linné, 1767), Cape Florida Key, Biscayne, Florida, MCZ No. 155173 — aperture view. X 5/6.

Figure 19, *Littorina littorea* (Linné, 1758), Hadley Harbor, Elizabeth-Islands, Massachusetts, Sta. P-0610-65, Systematics-Ecology Program, Marine Laboratory — aperture view. X 1.

Figure 20. *Lunatia triseriata* (Say, 1826), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-247-65, Systematics-Ecology Program, Marine Biological Laboratory — aperture view. X 2.

Figure 21. *Murex brevifrons* (Lamarck, 1822), Mayaguez, Puerto Rico, coll. M. R. Carriker, Systematics-Ecology Program, Marine Biological Laboratory — aperture view. X 1/3.

Figure 22. *Nassarius trivittatus* (Say, 1822), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-0605-65, Systematics-Ecology Program, Marine Biological Laboratory – aperture view. X 2.

Figure 23. *Nerita plexa* (Dillwyn, 1817), Mauritius, MCZ No. 139024 — aperture view. X 1/3.

Figure 24. *Oxynoe viridis* (Pease, 1864), from Pease coll., MCZ (uncat.) aperture view. X 2 1/3.

Figure 25. *Polinices duplicatus* (Say, 1822), Provincetown, Massachusetts, MCZ (uncat.) — aperture view. X 1 1/3.

Figure 26. *Siphonaria alternata* (Say, 1826), Bermuda, coll. Bryant, 1903, MCZ No. 24213 — top view (exterior). X 1 1/3.

Figure 27. *Succinea ovalis* (Say, 1817), bank of Grand River, W. Bridge Street Ferry, Ottawa County, Michigan, MCZ No. 166697 — aperture view. X 2.

Figure 28. *Turritella terebra* (Lamarck, 1822), Manila Bay, near Cavite, Luzon, Philippine Islands, MCZ No. 138070—aperture view. X 1/3.

Figure 29. *Umbraculum indicum* (Lamarck, 1819), Hawaii, C. B. Adams coll., MCZ No. 1173 — top view (exterior); 29A, side view. X 1/3.

Figure 30. *Urosalpinx cinerea* (Say, 1822), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-0612-65, Systematics-Ecology Program, Marine Biological Laboratory — aperture view. X 4 2/3.

PLATE I (Continued)

Figure 31. *Viviparus georgianus* (I. Lea, 1834), Lake Woodruff near Fick Island, Volusa County, Florida, MCZ No. 186800 — aperture view. X 2/3.

AMPHINEURA

Figure 32. *Chaetopleura apiculata* (Say, 1830), Hadley Harbor, Elizabeth Islands, Sta. P-7-63, Systematics-Ecology Program, Marine Biological Laboratory — top view (exterior). X 2.
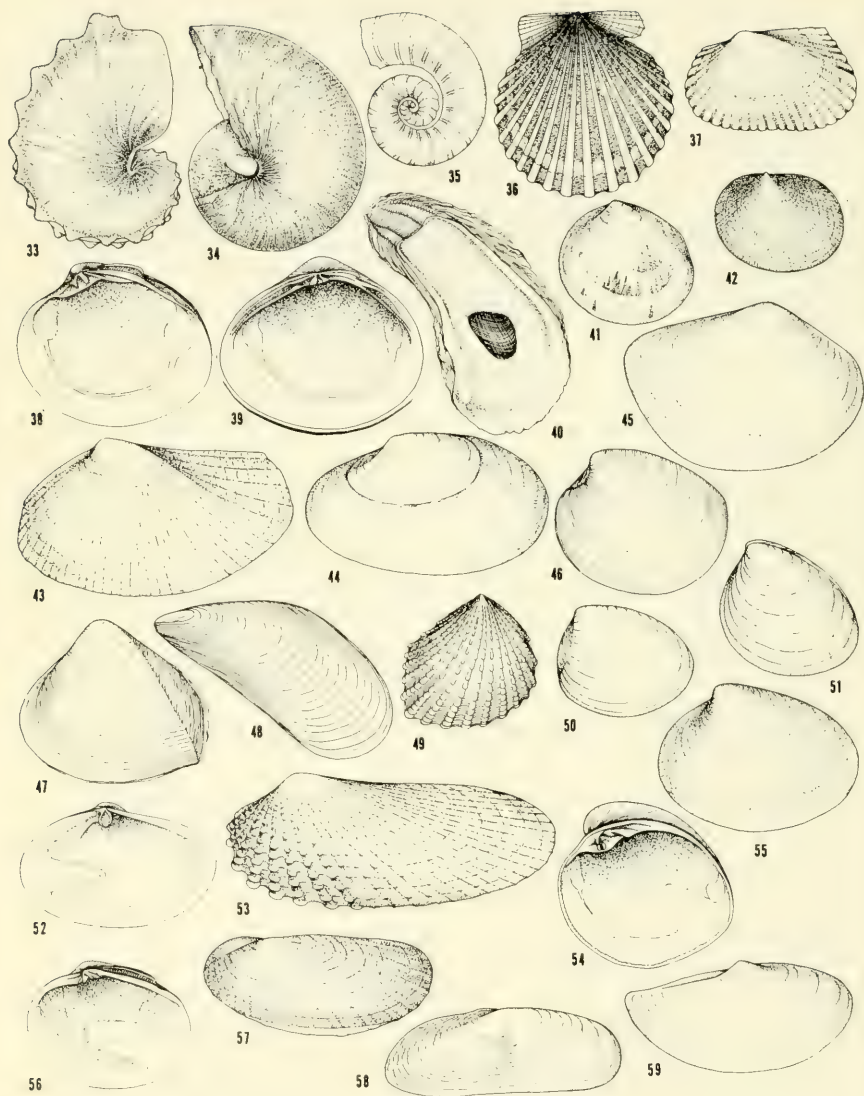
PLATE II

CEPHALOPODA

Figure 33. *Argonauta hians* (Dillwyn, 1817), southern Tropical Atlantic, MCZ (uncat.) — side view of egg case. X 1/3.

Figure 34. *Nautilus pompilius* (Linné, 1758), Southwest Pacific Ocean, MCZ (uncat.) — side view. X 1/3.

Figure 35. *Spirula spirula* (Linné, 1758), Saint Kitts Island, B.W.I., MCZ (uncat.) — side view. X 1 1/3.

PELECYPODA

Figure 36. *Aequipecten irradians* (Lamarck, 1819), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-65-64, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, right valve. X 1.

Figure 37. *Anadara transversa* (Say, 1822), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-77-64, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 9 1/3.

Figure 38. *Arctica islandica* (Linné, 1758), Georges Bank, Massachusetts, MCZ No. 14325 — interior view, right valve. X 1/3.

Figure 39. *Corbicula consobrina* (Cailliand, 1827), Nile River, Egypt, MCZ No. 14676 — interior view, right valve. X 1 1/3.

Figure 40. *Crassostrea virginica* (Gmelin, 1791), Holding Tank, Supply Department, Marine Biological Laboratory, Woods Hole, Massachusetts — interior view, right valve. X 1/3.

Figure 41. *Laevicardium mortoni* (Conrad, 1831), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-141-64, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 8 1/3.

Figure 42. *Limopsis compressus* (Dall, 1908), off Salina Cruz, Mexico, 1020-1240 m. coll. R. H. Parker, MCZ (uncat.) — exterior view, left valve. X 2/3.

Figure 43. *Lyonsia hyalina* (Conrad, 1831), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-200-64, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 5.

Figure 44. *Macoma tenta* (Say, 1834), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-91-64, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 2 1/3.

Figure 45. *Malletia,* species "M," Atlantic Abyssal Plain, 4970 m, coll. H. L. Sanders, in coll. at Woods Hole Oceanographic Institution — exterior view, left valve. X 2 1/3.

PLATE II (Continued)

Figure 46. *Mercenaria mercenaria* (Linné, 1758), Holding Tank, Supply Department, Marine Biological Laboratory, Woods Hole, Massachusetts — exterior view, left valve. X 2 1/3.

Figure 47. *Mulinia lateralis* (Say, 1822), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-0902-65, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 2 1/3.

Figure 48. *Mytilus edulis* (Linné, 1758), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-148-64, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 2 2/3.

Figure 49. *Neotrigonia margaritacea* (Lamarck, 1803), New South Wales, Australia, from coll. of A. L. McAlester, Yale University — exterior view, right valve. X 2/3.

Figure 50. *Nucula proxima* (Say, 1822), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-92-64, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 2 1/3.

Figure 51. *Nucula proxima* variety *truncula* (Dall, 1898), Buzzards Bay, Massachusetts, Sta. "R," coll. H. L. Sanders, Woods Hole Oceanographic Institution — exterior view, left valve. X 4 2/3.

Figure 52. *Periploma leanum* (Conrad, 1831), Martha's Vineyard, Chappaquidick Island, Massachusetts, MCZ No. 192934 — interior view, left valve. X 1/6.

Figure 53. *Petricola pholadiformis* (Lamarck, 1818), Black Beach, West Falmouth, SEP-949, George M. Gray Museum, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 2 1/3.

Figure 54. *Pitar cordata* (Schwengle, 1951), off Port Aransas, Texas, 80 m, MCZ No. 194372 — interior view, right valve. X 2/3.

Figure 55. *Pitar morrhuana* (Linsley, 1845), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-189-64, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 2 1/3.

Figure 56. *Saxidomus nuttalli* (Conrad, 1837), Gulf of Georgia, British Columbia, Canada, MCZ No. 5235 — interior view, right valve. X 1/3.

Figure 57. *Solemya velum* (Say, 1822), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-225-65, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 2 1/3.

Figure 58. *Tagelus divisus* (Spengler, 1794), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-21-63, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 2 1/3.

Figure 59. *Yoldia limatula* (Say, 1831), Hadley Harbor, Elizabeth Islands, Massachusetts, Sta. P-267-65, Systematics-Ecology Program, Marine Biological Laboratory — exterior view, left valve. X 2 1/3.

TABLE I

DISTANCE BETWEEN GENERA WHEN CONSIDERED AS POINTS IN FACTOR SCORE SPACE

GASTROPODA (8 DIMENSIONS)

PELECYPODA (9 DIMENSIONS)

Top (GASTROPODA) column genera: LITTORINA, UROSALPINX, CREPIDULA, LUNATIA, NASSARIUS, OXYNOE, ACHATINELLA, SIPHONARIA, CAVOLINA, HELIX, SUCCINEA, ARCHITECTONICA, ASTRAEA, CYPRAEA, FISSURELLA, ACMAEA, POLINICES, APLYSIA, UMBRACULUM, BULLA, JANTHINA, DOLABELLA, HALIOTIS, EPITONIUM, NERITA, VIVIPARUS, MELANELLA, HELISOMA, MUREX, TURRITELLA, AKERA

Left (GASTROPODA) row genera: PLANORBIS, AKERA, TURRITELLA, MUREX, HELISOMA, MELANELLA, VIVIPARUS, NERITA, EPITONIUM, HALIOTIS, DOLABELLA, JANTHINA, BULLA, UMBRACULUM, APLYSIA, POLINICES, ACMAEA, FISSURELLA, CYPRAEA, ASTRAEA, ARCHITECTONICA, SUCCINEA, HELIX, CAVOLINA, SIPHONARIA, ACHATINELLA, OXYNOE, NASSARIUS, LUNATIA, CREPIDULA, UROSALPINX, LITTORINA

Right (PELECYPODA) row genera: NEOTRIGONIA, TAGELUS, PERIPLOMA, CORBICULA, SAXIDOMUS, CRASSOSTREA, ARCTICA, LYONSIA, MALLETIA, MACOMA, LIMOPSIS, MERCENARIA, PETRICOLA, AEQUIPECTEN, LAEVICARDIUM, MYTILUS, SOLEMYA, MULINIA, PITAR, YOLDIA, ANADARA

Bottom (PELECYPODA) column genera: ANADARA, YOLDIA, PITAR, MULINIA, SOLEMYA, MYTILUS, LAEVICARDIUM, AEQUIPECTEN, PETRICOLA, MERCENARIA, LIMOPSIS, MACOMA, MALLETIA, LYONSIA, ARCTICA, CRASSOSTREA, SAXIDOMUS, CORBICULA, PERIPLOMA, TAGELUS, NEOTRIGONIA, NUCULA

BREVIORA

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TABLE 2

AMINO ACID RATIOS PER 1000 TOTAL

	Chaeto-pleura Mantle	*Chaeto-pleura* Shell	*Spirula* Shell	*Sepia* Shell (cuttle bone)	*Loligo* Shell (pen)	*Nautilus* Shell	*Haliotis* Shell	*Nucula* Shell	*Mytilus* Shell	*Neopilina* Shell with periostracum	Portunid Crabs* Uncalcified Integument	Portunid Crabs* Calcified Integument
OH-Proline	58	--	--	--	--	--	--	--	--	--	--	--
Aspartic Acid	50	128	130	116	74	83	164	69	112	61	102	81
Threonine	21	60	57	43	40	14	22	16	16	23	63	57
Serine	35	68	144	98	52	110	110	73	98	59	69	91
Glutamic Acid	69	98	117	73	44	49	59	35	38	39	113	94
Proline	99	93	71	107	151	8	41	25	74	61	63	103
Glycine	264	131	103	120	131	338	200	453	289	376	133	113
Alanine	136	88	66	106	158	231	144	120	241	50	68	115
Cystine [half]	3	14	31	28	0.2	6	0.4	13	11	2	--	3
Valine	48	45	39	40	65	16	25	20	27	74	44	65
Methionine	7	12	10	10	10	4	5	20	6	15	6	7
Isoleucine	28	30	25	20	24	15	15	12	16	41	37	28
Leucine	65	52	43	51	69	25	26	33	48	98	47	49
Tyrosine	0.6	31	25	60	68	12	33	4	20	1	28	25
Phenylalanine	19	37	27	21	26	54	30	48	17	29	46	30
OH-Lysine	4	--	21	--	16	0.4	0.4	0.7	--	0.3	--	3
Lysine	31	41	31	38	55	2	29	19	18	16	31	52
Histidine	0.4	11	4	49	8	0.4	7	11	5	5	34	26
Arginine	63	61	65	22	8	28	68	27	24	50	80	35
Protein / Hexosamines	45.96	0.98	2	2	2	30	13	274	219	160	1.53	.40

* Geometric means - 3 species uncalcified, 6 calcified. Protein/hexosamine arithmetic means.

TABLE 3

FACTOR SCORES. GASTROPODA.

(Shell Tissues)

Phylogenetic Relationships	Varimax Factors	Positive Loading / Negative Loading	1 GLY / GLU THR MET	2 VAL ISOLEU LEU	3 OHLYS LYS HIS TYR	4 PRO / ARG	5 / CYS	6 SER ALA / PHE	7 OHPRO	8 / ASP
?	SIPHONARIA		-0.8	-0.2	-0.6	-0.5	-0.3	-2.1	0.1	0.1
	HELISOMA		2.2	-0.4	0.3	0.7	0.9	0.2	-0.3	-1.7
?	PLANORBIS		0.6	0.9	5.2	0.9	-0.2	0.8	0.5	-0.3
	ACHATINELLA		1.8	2.0	-0.4	-0.7	0.6	-1.7	-0.3	1.2
	SUCCINEA		3.0	2.0	-0.4	0.8	0.2	-0.1	-0.7	1.4
	CAVOLINA		-0.3	-0.2	0.2	2.5	0.1	-0.1	0.0	-0.3
	UMBRACULUM		-0.6	-0.4	-0.3	1.7	-0.2	0.0	-0.4	-0.5
	ARCHITECTONICA		-0.2	-0.1	0.2	1.0	0.1	0.1	-0.2	-0.5
	AKERA		-0.1	0.6	-0.5	-0.0	0.4	0.2	1.4	-0.2
	OXYNOE		-0.4	0.4	-0.5	0.3	0.4	-0.2	5.6	-0.1
	APLYSIA		-0.6	1.1	-0.4	-1.2	-0.1	-0.2	-0.2	-0.6
	BULLA		-0.0	1.0	0.0	-0.2	-0.1	0.9	-0.6	-1.3
?	DOLABELLA		0.4	-1.3	0.0	-0.1	0.4	-1.1	-0.6	-3.9
	EPITONIUM		-1.0	-0.5	1.5	-0.7	0.5	0.3	-0.3	1.4
	JANTHINA		-1.0	-0.1	0.0	-0.3	0.4	-0.5	-0.3	0.4
	TURRITELLA		-0.4	-0.7	0.2	0.3	0.1	0.3	-0.3	-0.1
	MUREX		-0.8	-0.7	1.8	-2.1	0.9	-0.8	-0.6	0.7
	UROSALPINX		-0.8	0.7	-0.3	-0.9	0.1	-0.5	-0.3	0.3
	NASSARIUS		-0.3	1.4	-0.2	0.3	0.1	-0.3	-0.2	0.1
	COLUS		0.2	0.8	-0.9	1.0	-0.6	0.5	-0.1	-1.4
	MELANELLA		0.9	-1.3	-0.5	-0.6	-0.1	2.2	0.2	1.1
	POLINICES		0.2	-0.7	-0.5	0.7	0.2	1.2	0.1	0.7
	LUNATIA		-0.1	0.8	-0.4	-1.3	0.4	0.2	-0.2	-0.4
	LITTORINA		-1.0	0.4	-0.2	-0.2	0.3	-0.5	-0.3	-0.2
	CYPRAEA		-1.2	0.4	-0.6	-0.1	-0.1	-0.3	-0.1	0.5
	CREPIDULA PLANA		-0.6	0.8	-0.7	0.2	-0.1	0.2	-0.6	-0.3
	CREPIDULA FORNICATA		-0.6	1.6	-0.3	-1.1	-0.8	0.1	-0.6	-0.5
?	FISSURELLA		-0.8	-0.5	0.5	0.2	-0.1	-0.7	-0.1	0.2
?	ACMAEA		-0.7	0.9	-0.2	0.4	0.1	0.2	-0.3	-0.2
	VIVIPARUS		2.3	-1.6	-0.1	0.4	0.5	-2.9	0.1	1.3
	NERITA		-0.8	-1.0	0.6	-0.4	0.3	-1.1	0.2	0.6
	ASTRAEA		1.0	-1.6	-0.7	-1.8	0.0	0.9	0.5	-0.2
	HALIOTIS		1.2	-1.8	0.0	-1.5	0.1	0.0	0.2	-1.3

TABLE 4

FACTOR SCORES. PELECYPODA.

(Shell Tissues)

Phylogenetic Relationships	Varimax Factors	Positive Loading	1 THR GLU PRO	2 VAL	3 LYS HIS ARG	4 ALA	5 ISOLEU LEU VAL HIS	6 TYR	7 OHLYS	8	9
		Negative Loading	GLY	SER		MET				ASP	PHE
PITAR.			0.3	0.3	0.2	-0.5	-0.7	-1.1	-0.4	-1.9	-0.9
MERCENARIA			2.4	-1.0	1.8	0.2	-1.1	1.8	-1.3	1.4	0.4
SAXIDOMUS			1.3	-1.1	-0.4	0.5	0.5	0.3	-0.2	-0.9	-1.4
PETRICOLA			0.5	-0.6	-1.0	-1.1	1.5	1.9	-0.2	1.0	-0.9
LAEVICARDIUM			2.2	0.5	-1.0	-0.8	0.4	-1.2	-1.5	1.6	2.0
MACOMA			-0.6	0.1	-0.4	-1.8	-0.4	-0.9	-0.1	1.6	0.7
MULINIA			0.1	0.7	-0.5	-0.2	-0.5	0.1	-0.1	-1.0	0.3
TAGELUS			0.8	-0.6	-0.5	0.1	-1.0	0.0	0.0	0.3	-0.4
CORBICULA			0.7	-0.3	-0.3	0.4	-0.8	1.6	-0.3	-0.7	0.9
ARCTICA			2.9	0.3	-0.5	1.1	-0.1	1.1	4.8	-0.4	0.0
AEQUIPECTEN			-0.6	-3.1	-0.5	-0.6	-0.6	-0.7	0.1	-1.5	1.9
CRASSOSTREA			-0.9	-2.1	0.3	-0.5	-1.2	1.2	-0.2	-0.6	1.2
MYTILUS			-0.8	-0.3	-0.6	3.0	0.5	0.0	-0.4	0.4	1.0
ANADARA			-0.5	1.3	1.7	0.1	-0.2	1.4	0.1	-0.4	-0.1
LIMOPSIS			0.1	-1.2	2.0	-0.8	-0.5	-1.8	-0.3	0.8	-2.3
NEOTRIGONIA			-1.1	-0.8	0.1	1.5	0.0	-1.0	1.2	0.7	0.2
PERIPLOMA			-1.2	-0.6	0.6	-0.8	-0.4	2.6	-0.4	0.9	-1.8
LYONSIA			-1.1	-0.6	0.2	1.1	0.0	0.3	0.3	1.2	-1.4
NUCULA			-0.6	0.1	0.2	0.7	-0.7	-0.7	0.0	1.0	-0.9
SOLEMYA			-0.9	1.3	-0.8	-1.1	0.2	-0.7	0.2	0.8	-0.6
MALLETIA			0.5	1.0	0.9	0.2	-1.5	-0.3	-0.5	0.6	0.4
YOLDIA			0.9	1.2	0.2	0.6	-0.6	-0.1	0.2	-1.2	-0.2

TABLE 5

AMINO ACID RATIOS PER 1000 TOTAL

	Nerita	Viviparus	Archi-tectonica	Siphonaria	Helisoma	Planorbis	Succinea	Achatinella
	Marine	Fresh Water	Marine	Marine	Fresh Water	Fresh Water	Land	Land
OH-Proline	--	--	--	--	--			--
Aspartic Acid	103	48	132	133	143	98	11	47
Threonine	50	11	57	46	23	56	11	16
Serine	70	34	88	58	74	61	37	25
Glutamic Acid	114	26	114	103	69	91	9	29
Proline	85	54	90	82	39	59	57	50
Glycine	116	519	159	116	380	114	526	411
Alanine	108	16	81	74	62	88	19	30
Cystine [half]	2	8	15	16	3	21	4	4
Valine	48	17	40	63	38	61	83	78
Methionine	26	11	19	30	3	10	1	2
Isoleucine	23	26	36	35	15	37	50	64
Leucine	68	59	76	76	50	56	121	110
Tyrosine	23	46	14	15	42	67	28	11
Phenylalanine	44	95	19	74	17	--	16	74
OH-Lysine	1	--	3	--	1	16	--	0.3
Lysine	36	15	26	34	25	82	12	39
Histidine	16	9	6	1	1	34	0.2	0.2
Arginine	67	6	25	45	15	48	6	9
Protein Hexosamine	66	1060	22	23	11	n.d.	77	82